ON HAEMOGLOBIN METABOLISM IN MALARIAL FEVER

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I. INTRODUCTION

The following studies were carried out at the suggestion of Major Ross on some of the thirty-three cases of malaria which have occurred since January, 1910, at the Royal Southern Hospital, Liverpool, and which have already been reported upon by him and Dr. David Thomson in their paper on 'Enumerative Studies on Malarial Fever.'† The funds for the research were allotted to the Liverpool School of Tropical Medicine by the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office). Much of the work was done in the laboratory of Professor Benjamin Moore, University of Liverpool, to whom we are indebted for advice and assistance.

The object of the research was to ascertain the fate of the haemoglobin, which is well known to be set free from the red corpuscles in malarial fever, and to measure its amount, in the ultimate hope that more light might thus be thrown on various problems connected with malaria, and especially with blackwater fever. The daily details of my findings are incorporated in the tables of the paper just mentioned, in order that they may be read in connection with the marked fall, recorded by the haemoglobinometer, in the haemoglobin of the circulating blood during the pyrexial periods.

II. PRELIMINARY

At first a careful search of the urine was made during and after the malarial paroxysms in eight patients. No trace of haemoglobin or of haemin or other immediate derivatives of haemoglobin was found, except in two cases, where a few red blood corpuscles were

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found associated with oxaluria. Hyaline and granular casts and oxalate crystals were present in the urine for a few days after the paroxysms in five of these cases (Nos. 10 to 14 inclusive). A greater measure of success was attained when attention was directed to the more remote products of haemoglobin breakdown; of these the iron-free pigment nucleus alone held out prospects of successful quantitative work, since the iron itself is known to be in a large measure retained in the body and the protein portion is merged in the general protein metabolism. Haemoglobin, whether arising by haemolysis in the body or experimentally introduced, is withdrawn from the circulation and broken down into its constituents by the liver, and the pigment moiety is excreted into the intestine as bile pigment. When amounts larger than about 0·3 gram of haemoglobin per kilo body weight are suddenly introduced into the circulating blood, some part may be excreted by the kidneys, and Barratt and Yorke* have shown that the haemoglobinuria varies with the degree of haemoglobinaemia.

The bile pigment leaves the body for the most part as urobilin in the urine and the faeces.

Since the relationship of haemoglobin and urobilin is known with fair accuracy, it is possible to calculate theoretically the amount of urobilin which would be yielded, if all the haemoglobin of a normal man could be transformed into that substance; or conversely what amount of haemoglobin must have been destroyed to give rise to a known excretion of urobilin. A man of 60 kilogrammes with a normal amount of red corpuscles, etc., would yield about 30 grams of urobilin from the haemoglobin (750 grams) of the circulating blood and about 20 grams more from the haemoglobin of his muscle and from pigment in the liver and bile passages.

III. URINARY UROBILIN

(*a*) The work of Hoppe-Seyler, Hildebrandt, and others has shown that urobilin is present only in traces in normal urines, but that it is increased in pyrexia, anaemias, hepatic disorders and internal haemorrhage. The excretion is from 0·01 to 0·1 gram per diem in the urine of healthy adults, and in pyrexias, etc., it rises as high as 0·5 gram per diem. Occasionally higher figures have been

* Annals of Trop. Med. and Parasit., Vol. III, No. 1, Section V.

recorded, 0·8 gram in scarlet fever, 2·02 gram in perityphlitis and 2 grams in malaria.

After trials of various methods, the method of quantitative determination adopted for the purpose of this work was to determine the dilution of the urine required to just render invisible the spectrum of urobilin, when examined by a spectroscope in a layer of 15 mm. thick. The spectroscope was standardised with solutions of purified urobilin, and the readings appear to be reasonably accurate except that, when only a low concentration of urobilin is present, they are too low. The spectroscopic results were checked in many instances by the more delicate fluorescence tests.

By this method I failed after repeated trials to find urobilin in any specimen of urine from healthy adults; amounts varying from 0·04 to 0·25 grams per diem were found in various pyrexial diseases and from 0·02 to 0·06 gram per diem in cases of anaemia, intermuscular haemorrhage and hepatic disease. In a case of Malta fever (temp. 104° F.) and in a case of acute suppurative hepatitis (probably dysenteric) (temp. 104° F.) 0·3 and 0·31 gram per diem were found respectively.

(*b*) In five cases of *simple tertian malaria* (*P. vivax*) the amount of urobilin found in the urine in the period of pyrexia and the days following was never greater than the average excretion in diseases such as pneumonia with an equal degree of pyrexia, and in Case 3, indeed, was scarcely perceptibly increased from the normal limits:—

Maximal excretion in twenty-four hours (Case 4) 0·19 gram.

Maximal total excretion in seven days after pyrexia (Case 4) 0·42 gram.

(*c*) *In malignant tertian malaria* (*P. falciparum*) a different picture is obtained; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes; the normal level of excretion is reached in seven to ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20), and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19); and in these cases marked haematoporphyrinuria was several times noted (Cases 17, 18, 19). (Haematoporphyrinuria was also noted on one occasion in Cases 20 and 23.)

Average excretions of urinary urobilin in malignant tertian malaria (Cases 10, 12, 17, 19, 20).

Maximum excretion in twenty-four hours (Cases 20 and 17) 0·2 to 0·30 grams.

Maximum total excretion in seven days (Cases 17 and 12) 0·6 to 0·7 gram.

The amount of urobilinuria (even if considered in comparison with body weight) bore no apparent relation (1) to the degree of pyrexia; (2) to the fall of the haemoglobinometer readings during the pyrexia; or (3) to the absolute numbers of parasites present in the blood.

(*d*) *Special high excretion in malignant tertian malaria.* In four cases the excretion of urobilin was so much higher that they will be mentioned separately and in more detail. In Cases 11, 13, and 23, the patients were young, and large numbers of parasites were present with correspondingly severe symptoms; in Case 21 the patient was older and, though the parasites were much less numerous and the pyrexia less marked, he was markedly prostrated by his attack.

I may mention that the urine in these cases became extremely dark on standing in daylight, after acidification, and that the band of urobilin remained visible in two instances until the urine was diluted to one hundred and fifty volumes. Cases 13 and 21 were constipated at the time the high-days' output took place.

I give in each instance the maximal twenty-four hours' output and the total excretion for seven days, and have also determined the percentage relation between these amounts and the normal circulating haemoglobin of the patients.

Number of Case	11	13	21	23
Weight in kilos.	60	54	72	50
Maximal 24 hours' excretion—				
As grams of Urobilin	2·31	0·9	1·73	2·0
As percentage of total Hbg.	8·1	3·5	5·0	9·5
Total 7 days' excretion—				
As grams of Urobilin	3·9	1·7	2·38	2·26
As percentage of total Hbg.	14·0	6·8	7·2	11·2
As corpuscles per mm.³ of blood	700,000	340,000	360,000	560,000
Fall in Haemoglobin as recorded by Haemoglobinometer	?	?	30 %	20 %
Number of parasites per mm.³ of blood	64,000	300,000	36,000	50,000

The figures are very high indeed, and equal, in some instances, any figure that I have found recorded by other observers; their special interest is that they show the destruction and elimination of a very large proportion of the haemoglobin of the circulating blood; I propose, however, to leave the question of urinary urobilin at this stage and proceed to consider the elimination by the bowel. As will be seen later urinary urobilin would appear to be simply a leakage—rarely an important one—from the other channel, and it has only been alluded to in consideration of the great attention that has been paid to it in the past, and since in the present instance, it was largely the promise held out by the results obtained in Cases 11 and 13 in the early days of January, that led to my persisting in further work along these lines.

Note.—Since writing this paper I have become acquainted with De Jonge's* results, he found urobilin in malarial urines in amounts of from 0·05 to 0·10 gram per diem, and this frequently rose as high as 1 gram and occasionally even to 4 grams. The difference may be accounted for by the fact that his cases, unlike mine, were frequently treated with quinine, which he found to increase the urobilin output. He found however lower outputs in malignant tertian malaria than in benign tertian, a result very different from mine.

IV. FAECAL UROBILIN

(*a*) It would be out of place in the present paper to discuss at any length the methods of extraction and estimation of urobilin from the faeces, but perhaps I may be pardoned for alluding to a few points of interest.

After considerable controversy it has been shown that urobilin of identical quantitative composition can, by various processes, be prepared from faecal and urinary sources. It would appear, however, that much of the urobilin in the faeces is present in modified forms (possibly in part in union with metallic salts) since it has a fluorescence and occasionally a spectroscopical appearance somewhat different from that of urinary urobilin; on one or two occasions I was very much puzzled by obtaining a solution much

* Geneesk. Tijdschr. v. Nederl.-Indie, Deel XLIV, p. 435, 1904.

more purple in colour than usual, and with a slightly altered band, and it was only after considerable expenditure of time that I discovered that part of the urobilin was present as its mercury compound owing to the administration of calomel.

Another variation which gave rise to great difficulty is that the urobilin, obtained by the extraction of the faeces with acidulated water, proves very refractory at different stages in the process of purification and separation, described by Garrod and Hopkins—indeed we have as yet failed to get a reasonably pure product from the faeces and so possibly the figures obtained may later require revision.

The urobilin was extracted by repeated shaking with large amounts of water, acidulated with dilute sulphuric acid, and the filtrates were fully exposed to daylight for some time, before being estimated by the same method as was adopted for urinary urobilin; this solvent has the advantage that it does not take up bile pigment or chlorophyll to any extent, and only on one occasion (later extracts of a case of blackwater fever) did I find any definite band other than that of urobilin, though other writers seem to have found such extra bands very frequently when using different solvents.

Another point of interest is that in such literature as I have discovered on faecal urobilin, the writers seem to convey the impression that it is present in small amounts and requires somewhat delicate tests for its identification. Only in McMunn's papers did I find descriptions of solutions comparable in colour and spectroscopic appearances to those with which I had become acquainted, and in which, as a general rule, a very high dilution was necessary before the general absorption was overcome; and the urobilin band was the only one ever distinguished save in the instance referred to in the last paragraph, when a band was also present embracing the D line, probably due to haematoporphyrin.

From Case 23, for example, nearly two litres of acidulated extract were obtained in which there were visible:—

1.	Acid Urobilin Band	to a dilution of		100	volumes.
2.	Mercury „	„	...	120	„
3.	Pink colour of mercury urobilin	„	...	800	„
4.	Fluorescence with Zinc Chloride	„	...	300	„

In another instance (blackwater fever case) from the same stool I obtained:—

4,000 c.c. of extract with Acid Urobilin band visible to 400 dilutions and Fluorescence (Zinc Acetate) to 1,600 dilutions.

7,400 c.c. of extract with Acid Urobilin band visible to 200 dilutions and Fluorescence (Zinc Acetate) to 1,000 dilutions.

3,000 c.c. of extract with Acid Urobilin band visible to 40 dilutions and Fluorescence (Zinc Acetate) to 600 dilutions.

In all 3,200 litres of fluid showing Urobilin band, or 15,600 litres of fluid showing clearly green Fluorescence with Zinc Acetate, in each case in test-tubes fifteen millimetres in diameter.

The only quantitative figures I have found in the literature for faecal urobilin are:—

0·021 to 0·029 gram per diem in cases of Chlorosis.
0·153 to 0·92 „ „ „ Pernicious Anaemia.

My own results included the following:—

Stools of healthy adults on full diet	...	0·03 to 0·06 gram per diem.
Pneumonia (temp. 104°F., milk diet)	...	0·08 „ „
Malta Fever (temp. 104°F., milk diet)	...	0·4 „ „
Anaemic Girl (meat diet)	...	0·04 to 0·13 „ „
Intramuscular Haemorrhage (meat diet)		0·12 to 0·13 „ „
Amoebic Suppurative Hepatitis (temp. 103°)	...	0·12 to 0·13 „ „
Chronic Malarial Anaemia	... ...	0·15 „ „

(*b*) *Faecal urobilin in benign tertian malaria* (*P. vivax*). In three cases of this disease the excretion was found to be comparable to that found in other pyrexial diseases (in a fourth, Case 8, it was much higher, but was only taken on one day).

Case 6—Maximum day's excretion	... ...	0·49 gram.
Total excretion in 7 days	... ...	1·22 „
Control stool (13th day, meat diet)	... ...	0·02 „
Case 7—Maximum day's excretion	... ...	0·53 „
Total excretion in 7 days	... ...	1·30 „
Control stools (10th and 11th days, meat diet)	...	0·07 and 0·05 gram.
Case 8—Only one stool examined (4th day)	... ...	1·12 gram.

These excretions represent in Cases 6 and 7 about 4 per cent. of the patients' total circulating haemoglobin, the haemoglobinometer shows a much greater fall (20 per cent. in Case 7).

(*c*) *Faecal urobilin in malignant tertian malaria* (*P. falciparum*). Eight cases of disease have been examined for faecal urobilin, and in practically every instance a higher output was shown.

In five instances, only isolated stools shortly after the paroxysm were examined, and usually also a control stool, when the patient was again convalescent and on meat diet.

Case Number	Day of disease	Faecal Ub. (grams)	Faecal Ub. (per cent. of Hbg.)	Fall of Haem. (Haemoglobinometer)
17	4	1·13	4	20
	13 (relapse)	0·45	1·6	10
	21 (control)	0·28	1·0	—
24	20 (relapse)	0·8	2·4	5
	16 (control)	0·14	0·42	—
25	1	0·12	—	—
	7	0·51	—	—
28	2	1·13	3·5	5

In the two remaining cases the faecal excretion was followed throughout the attack, save for occasional unavoidable losses. The history of these cases (21 and 23) has already been alluded to in the section dealing with urinary urobilin.

In Case 23 the urobilin output during convalescence and on meat diet was from 0·01 to 0·02 gram per diem, and during his pyrexia this rose to as much as 1·73 grams.

In the first seven days after the onset of pyrexia his total urobilin output was 6·4 grams, representing no less than 25 per cent. of his total haemoglobin (of this amount 4·3 grams appeared in the faeces, 2·1 grams in the urine). His haemoglobinometer reading fell 20 per cent. and recovered 6 per cent. in the last two days of the week; the number of red blood cells destroyed (calculated from urobilin excretion) must have been about 1,250,000 per mm.^{3}, while the maximum number of parasites observed was only 50,000 per mm.^{3}.

In two subsequent relapses his urobilin output was very much lower (about 1·5 grams), but in the first of these especially a much more marked fall occurred, as shown by the haemoglobinometer, and the recovery was much slower, suggesting possibly that the reserves of haemoglobin precursors had become exhausted.

Case 21 had a rather higher output of urobilin during convalescence, amounting to 0·15 gram per diem, but after pyrexia it

reached the high figure of 4·1 grams (following three days' constipation). Some stools were unfortunately lost, but his total urobilin output (urinary and faecal) amounted to 6·8 grams, which represents 20 per cent. of his total normal haemoglobin.

The haemoglobinometer readings fell 25 per cent., of which 15 per cent. was regained within a week. His urobilin output corresponds to a destruction of 1,000,000 corpuscles per mm.^{3}, while the maximum number of parasites seen in the peripheral blood was 35,000 per mm.^{3}.

V. SUMMARY

There can therefore be little doubt that, in malignant tertian (*P. falciparum*) infection, the excretion of haemoglobin derivatives is much higher than in other diseases with a similar degree of pyrexia; this excretion is accompanied by, and probably depends on, a fall in the haemoglobin present in the circulating blood, and would seem to demonstrate that the fall in the haemoglobinometer readings represents an actual destruction of red blood cells, not merely an alteration in the relative proportions of corpuscles and plasma in the peripheral blood.

In Cases 21 and 23, respectively, thirty-five and twenty-five corpuscles at least must have been destroyed for every parasite present, and this suggests either that the corpuscles are disintegrated mechanically (or as part of a protective mechanism) by the efforts of the spores to enter them, or else that the mother-parasite contains some haemolytic substance which is liberated on sporulation.

Finally I would like to draw attention to the relationship between the elimination by the urinary and faecal channels in these cases, and, indeed, in health and in other diseases. Only exceptionally does the urinary output approach the level of the faecal output, and yet practically all the quantitative work that has so far been done on urobilin, has been confined to the small and comparatively unimportant channel.

Before concluding I would like to refer to the eighth case of malignant tertian infection mentioned in dealing with faecal urobilin, and which, owing to its peculiar interest, is dealt with in a separate paper by Major Ross, Dr. Thomson and myself (p. 307).

When admitted to the hospital he had a fairly severe attack of malignant tertian malaria, and in the week following its onset he

eliminated about 3 grams of urobilin, the large majority in the faeces; this is equivalent to 10 per cent. of his total circulating haemoglobin, and corresponds with the elimination in ordinary cases of this infection; (under normal circumstances on full diet his faecal urobilin is about 0·18 gram per diem).

A week later he had another attack of fever, accompanied by haemoglobinuria—the complex known as blackwater fever—and in the first seven days of this attack he excreted the enormous amount of 33 grams of urobilin. In spite of marked urobilinuria during the period of haemoglobinuria only 1·25 grams of urobilin appeared in all in the urine, and of the remainder nearly the whole (29·3 grams) was present in one stool, of which some particulars were given in the preliminary remarks on faecal urobilin (this stool contained also a considerable amount of unaltered bile) Owing to his collapsed and unconscious state, several stools were lost, but his next three recoverable stools contained in all only some 0·8 gram of urobilin, (though the presence of diarrhoea may have led to an unduly low yield).

As will be remembered, a man of his weight (60 kilos) contains potentially some 30 grams from the haemoglobin of his circulating blood and probably about 20 grams more from other sources, so this patient seems to have suffered an almost total destruction of his circulating blood. The haemoglobinometer reading fell to 25 per cent., and so apparently some of the destroyed haemoglobin was rapidly replaced from other sources. The haemoglobinuria itself only accounted for the haemoglobin of 20 c.c. of blood, less than 0·5 per cent. of the total.

I do not propose to allude further to this case, but would like to indicate one other possible point of interest. Barratt and Yorke* state that haemoglobinuria arises in man, if a grade of haemoglobinaemia is attained exceeding that obtained by the solution of the haemoglobin of 0·25 per cent. by volume of wet red cells in the blood plasma; (roughly speaking, the volumes of plasma and corpuscles are equal in human blood, and so on this scale total haemolysis represents 100 per cent).

We have seen that in Cases 21 and 23 from 20 to 25 per cent. of the corpuscles were destroyed, and in these cases the period of pyrexia

* *Loc. cit.*, Section III.

(in which it appears legitimate to assume the haemolysis took place), occupied about thirty-six hours. So the haemoglobin of about 0·6 per cent. of red corpuscles was discharged into the plasma in every hour, if the haemolysis was spread evenly over the period of the crisis; and as the liver would need to remove this amount every hour from the whole blood to prevent the appearance of haemoglobinuria, any slight failure of the mechanism either from hepatic congestion or from all the haemolysis occurring in a very short period of time might serve to set up haemoglobinuria.

CONCLUSIONS

1. The main excretory channel for the pigment portion of the blood is the alimentary tract, and urinary urobilin represents only a small overflow from this source; the main elimination is in the faeces, and this has been largely neglected by previous observers, in favour of the urinary overflow; which, however, would seem to be of importance only as an indication of the absorptive activity of the intestine and almost negligible for quantitative purposes.

2. That the fall in the haemoglobinometer readings in malaria appears to represent an actual destruction of red corpuscles and elimination of their pigment by the normal excretory mechanism.

3. The haemoglobin breakdown during the pyrexia due to malignant tertian (*P. falciparum*) infection appears to be greater than in other pyrexial diseases or than in benign malaria (*P. vivax*), and may be many times greater than would be accounted for by the number of corpuscles infected by the parasites.

4. That as regards their effect of causing haemolysis, the parasites appear to be of different degrees of virulence in different individuals, and even in different paroxysms in the same individual (relatively at least, since the patent's resistance must be considered).

5. Normally the ordinary channels of excretion are capable of dealing with the free haemoglobin and rapidly remove it from the blood stream, and a very severe strain (over 25 per cent. of the total circulatory haemoglobin) can be sustained without their failure and the consequent onset of haemoglobinuria.